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Short communication

Promoting electrogenic ability of microbes with negative pressure

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HIGHLIGHTS

- Effect of negative pressure on the performance of electrode biofilm was firstly investigated.
- The MFCs in negative pressure generated power of 7 times higher than that in normal environment.
- Electrogenic ability of biofilm was promoted by strengthening attachment of EPS to electrode.

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ABSTRACT

Microbial fuel cells (MFCs) in negative pressure environment can provide an increase of 20% in voltage output and a maximum power generation of 7 times compared with that in normal environment. As showed by denaturing gradient gel electrophoresis, the culture in negative pressure environment for 30 days had little effect on diversity of bacterial communities. However, scanning electronic microscope indicated that negative pressure strengthened the attachment between extracellular polymeric substances and carbon felt electrode, it is the main reason for the increase in electrogenic ability of biofilm. The study suggested negative pressure-culture to be a method for promoting the performance of MFCs. The positive effect of negative pressure on the adhesion between biofilm and attachment materials also provides a potential method to improve the performance of biofilm-based environmental technology.

1. Introduction

Microorganisms can derive energy from oxidation of organics in wastewaters and simultaneously convert them into electricity via microbial fuel cells (MFCs) [1–3]. Therefore, MFCs were one of innovative technologies to conquer the environmental pollution with less energy supply [4]. To facilitate the application, previous works have been contributed to enhance the power generation of MFCs, such as changing architectural structure [5,6], isolating exoelectrogenic bacteria [7,8], investigating electron transfer mechanisms of exoelectrogenic bacteria [9,10].

Exoelectrogenic microbes can attach to electrode and form a layer of biofilm, which is the function center of respiring substances and producing current [11]. CO_2 is one of main metabolic products when biofilm is respiring organic substances. The dissolved CO_2 may acidify microenvironment of electrode surface and inhibit the metabolism of electrogenic ability of biofilm. In negative pressure condition, CO_2 will release from the bulk solution more easily due to the decrease of partial pressure in surrounding environment. Hence, a decrease in concentration of dissolved CO_2 may ease microbes from the suppression of acidification and increase the electrogenic ability of biofilm. However, no study has been conducted to investigate the effect of pressure on MFC performance.

In this study, MFC setups are placed in normal and negative pressure environments (i.e. −0.025, −0.05, −0.075 and −0.095 MPa) to investigate whether negative pressure can promote the performance. Voltage of the MFCs was recorded as a function of time, and discharge polarization and electrochemical impedance spectroscopy (EIS) were applied to analyze the reasons for the change in electricity production when the MFCs were in normal and negative pressure of −0.05 MPa environment. Denaturing gradient gel electrophoresis (DGGE) based on amplified 16S rRNA gene fragments was used to detect the dynamics of microbial communities on anode surface. Microscopy and scanning electronic microscopy (SEM) were used to investigate the effect of negative pressure on the morphology of biofilm.

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2. Materials and methods

2.1. Microbial fuel cell construction and operation

MFCs were constructed by joining two quadrate acrylic reactors (5 × 5 × 5 cm) containing a cation exchange membrane (4 × 4 cm) clamped between the two reactors. The anode was carbon felt (4 pieces, 4 × 4 cm each piece). The cathode was carbon cloth (8 × 8 cm) without any modification, and titanium wire and a 1000 Ω resistor were used to connect the circuit. The anode chamber was filled with 100 ml of medium containing about 7.35 mmol l^{-1} NaAc (1 g l^{-1}), 1 mmol l^{-1} $NaNO_3$ and 100 mmol l^{-1} KH_2PO_4 and inoculated with activated sludge (1 g dry weight) from a domestic wastewater treatment plant in Jimei, Xiamen City, China. The cathode chamber was filled with 100 ml of medium containing 50 mmol l^{-1} potassium ferricyanide and 100 mmol l^{-1} KH_2PO_4. The pH of bulk solution in anode and cathode was adjusted to 7.0.

The voltage across the resistor was recorded by a digital multimeter (Integra 2700 series equipped with 7700 multiplexer, Keithley Instruments Inc., USA). At the end of each cycle, 20 ml effluent in anode was replenished by 20 ml condensed medium (73.5 mmol l^{-1} NaAc, 10 mmol l^{-1} $NaNO_3$ and 200 mmol l^{-1} KH_2PO_4) and 40 ml effluent in cathode was replenished by 40 ml medium containing 50 mmol l^{-1} potassium ferricyanide and 100 mmol l^{-1} KH_2PO_4. After five repeatable cycles in normal environment, the MFCs were transferred to −0.025 MPa environment for testing. The negative pressure was sequentially set at −0.050, −0.075 and −0.095 MPa after three or four repeatable cycles. During the whole experiment, the temperature was controlled at 22 ± 2 °C.

2.2. Discharge polarization analysis

The discharge polarization analysis was measured by a battery test equipment (Model 2300, Maccor Inc., USA) using a constant resistor discharge procedure. The resistances were sequentially set at open circuit, 10000, 6000, 4000, 2000, 1000, 800, 600, 400, 200, 150, 100, 75, 50 and 25 Ω. The potentials of the cathode and the anode vs. Ag/AgCl as a function of time were individually recorded using a digital multi-meter (Integra 2700 series equipped with 7700 multiplexer, Keithley Instruments Inc., USA) interfaced to a personal computer for data collection.

2.3. Electrochemical impedance spectroscopy

The MFCs were analyzed by EIS before and after being cultured in negative pressure environment using an electrochemical workstation (AutoLab PGSTAT302N, Netherlands). Under open circuit condition, a three-electrode system and a two-electrode system were applied to analyze the resistance of the whole MFC and the cathode, respectively. In a two-electrode system, the anode served as working electrode and the cathode served as reference electrode and control electrode. In a three-electrode system, the cathode was the working electrode, the anode was the control electrode, and an Ag/AgCl was used as reference electrode. The frequency range was from 100 kHz to 10 mHz. The EIS data was simulated and fitted by Nova Version 1.7 (AutoLab, Netherlands).

2.4. DNA extraction and PCR

Microorganism samples were sequentially collected from the anodic electrodes just before moved to negative pressure environment, just after put it back in normal environment and three repeatable cycles after returned in normal environment. DNA was extracted and purified from the samples using a previously reported protocol [12]. Purified DNA was dissolved in 50 μl sterilized Milli-Q water (Millipore, USA), and 5 μl of DNA was used for agarose gel electrophoresis.

Bacterial universal 16S rRNA gene primer pair GC341f (5′- CGC CCG CCG CGC CCC GCG CCC GGC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG-3′) [13] and 517r (5′- ATT ACC GCG GCT GCT GG-3′) [14] was used to directly amplify partial 16S rRNA gene fragments from total DNA for DGGE analysis. Each 50 μl PCR reaction mixture contained 1 μl of template DNA (about 100 ng), 5 μl of 10 × buffer (Takara, China), 2 μl of 10 mmol l^{-1} dNTP mixture (Takara, China), 1 μl of 10 μmol ml^{-1} each primer (Sangon, China), 1 μl of 2.5 U μl^{-1} Taq DNA polymerase (Takara, China) and 40 μl of sterilized Milli-Q water. PCR amplification was run using the following cycling conditions: 5 min at 94 °C; 30 cycles with each cycle consisting of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C; followed by a final 7-min extension at 72 °C. 5 μl of PCR products were visualized on 1.5% agarose gel run at 10 V cm^{-1} for 25 min after mixing with 0.5 μl of 100× SYBR™ Green I. The left PCR products were stored at −20 °C before denaturing gradient gel electrophoresis (DGGE) analysis.

2.5. DGGE analysis

DGGE was carried out using a DCode™ Universal Detection System instrument and gradient former model 475 according to the manufacturer's instructions (Bio-Rad, USA). The denaturant solution was prepared as Muyzer et al. reported [13], and in this study, the acrylamide concentration in the gel was 8% and the denaturing gradient was 30–50%. Gels were run in 1× TAE buffer at 60 °C for 10 h at 100 V. Gels were stained with 1× SYBR™ Green I for 20 min and were visualized in the Ettan DIGE Imager system (GE, USA). DGGE analysis was performed more than three times to check the reproducibility.

2.6. Morphology analysis of biofilm

Two pieces of carbon felt (3 × 3 cm) were placed into two 250 ml beakers, respectively. Each beaker was filled with 225 ml medium containing 7.35 mmol l^{-1} NaAc, 1 mmol l^{-1} $NaNO_3$ and 100 mmol l^{-1} KH_2PO_4, and 25 ml of effluent from MFC M1 was inoculate into the beaker. The pH of medium was adjusted to 7.0. One beaker was placed into −0.05 MPa negative environments, and the other one was purged with N_2 for 1 h and sealed with film to maintain an anaerobic condition in normal environment. Every three days, the medium of 125 ml was replaced. After being cultured for 20 days, felts with biofilm were picked from the beakers and were inspected on an IX71 inverted microscope (Olympus, Japan). Four pieces of felt with biofilm (0.5 cm × 0.5 cm, two pieces from each) were carefully cut down from the carbon felts, and each was bathed for 12 h with 1 ml of glutaraldehyde (2.5%, w/v) to fix the bacteria cells. Then, all the felts were dried at 80 °C for 24 h. The dry felts were used to perform the scanning electron microscope on a field emission scanning electron microscopy (S-4800, Hitachi, Japan).

3. Results and discussion

3.1. Performance of MFCs

To investigate the effect of negative pressure on MFCs, we placed the two MFCs (M1 and M2) in normal environment and in −0.025, −0.05, −0.075 and −0.095 MPa negative pressure. Fig. 1 shows the voltage change of M1 connected an external resistance of 1000 Ω in different environments. After being cultured about 30

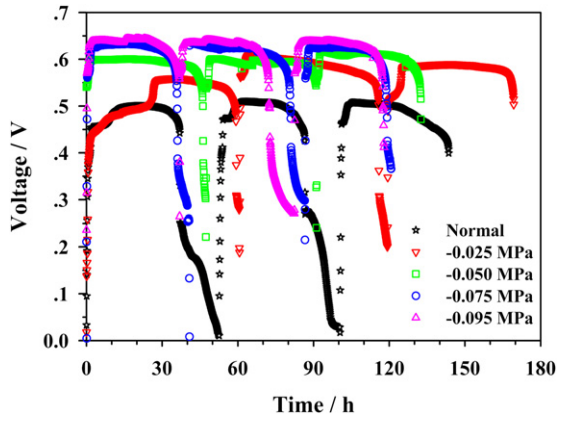

Fig. 1. Voltage change of an MFC being placed in different environments.

days in normal environment, M1 achieved a stable voltage output of 0.50 V for three repeatable batch modes. The voltage increased to 0.55 V after being placed in negative pressure of −0.025 MPa for 25 h and then increased to 0.60 V at the second cycle, which phenomena was not observed with the control. As the negative pressure decreased to −0.095 MPa, the voltage output increased to 0.64 V which was 0.14 V higher than that in normal environment. The voltage from M2 showed same reproducible results (Fig. S1). It can be concluded that a negative pressure environment enhanced rather than inhibited bacterial electrogenic ability by increasing the voltage output.

3.2. Polarization analysis

Polarization was employed to investigate how the negative pressure promoted the electrogenic ability of MFCs. In normal environments, the open circuit potential was 0.58 V, and the maximum power output was 0.22 mW when the resistance was set at 1000 Ω (Fig. 2a). After being cultured under negative pressure environment for 30 days, the open circuit potential increased to 0.69 V, and a maximum power output of 1.75 mW was achieved at external resistance of 150 Ω (Fig. 2b). The power output in negative pressure environment was 7 times higher than that in normal environment.

The potentials of anode were −0.25 and −0.35 V vs. Ag/AgCl when the MFC generated the maximum power in normal environment (Fig. 2c) and negative pressure environment (Fig. 2d), respectively. During the polarization measurement, the potential of cathode had little change. The result indicated that electrogenic ability of anode was improved by the culture in negative pressure environment. Although Portner et al. have reported that high vacuum couldn't kill bacteria [15], this study presented a positive effect of negative pressure on enhancing bacterial performance for the first time.

3.3. EIS analysis

From Fig. 2c and d, it shows that the anode is the key factor limiting the power output. Therefore, EIS was employed to study the chemical and physical processes in Fig. 3a, an equivalent circuit (Fig. 3b) was used for simulation of internal resistance [16]. The internal resistance of MFCs was the sum of R_s, R_c and R_a where they represented the ohmic resistance derived from ion and electron transport, the cathode resistance and the anode resistance, respectively. While MFC was in normal environment, the values for R_s, R_c and R_a were 3.4, 11.0 and 723.3 Ω, respectively. After being cultured in negative pressure for 30 days, the R_s, R_c and R_a of the MFC reduced to 3.2, 8.4 and 59.1 Ω, respectively. It seems that R_a is the major part of internal resistance and the changes in R_s and R_c are negligible compared with the reduce of R_a.

R_a consists of resistance derived from mass transfer (R_{con}) and from the slowness of the reactions taking place on the surface of the electrodes (R_{act}) [3]. The R_{con} is contributed by diffusion along concentration gradients, by migration when an electric field is present and convection in the presence of any form of agitation or forced motion [3]. In negative pressure environment, the dissolved

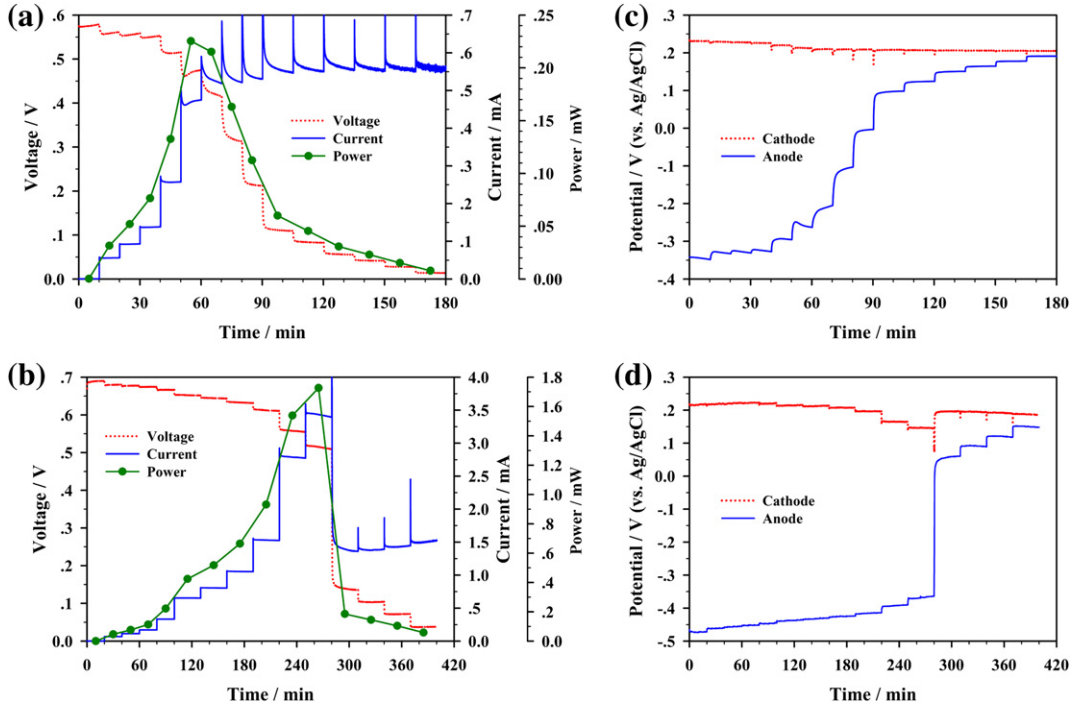

Fig. 2. Polarization curves in different environments via controlled external resistances. (a) and (c) in normal environment, (b) and (d) in negative pressure environment.

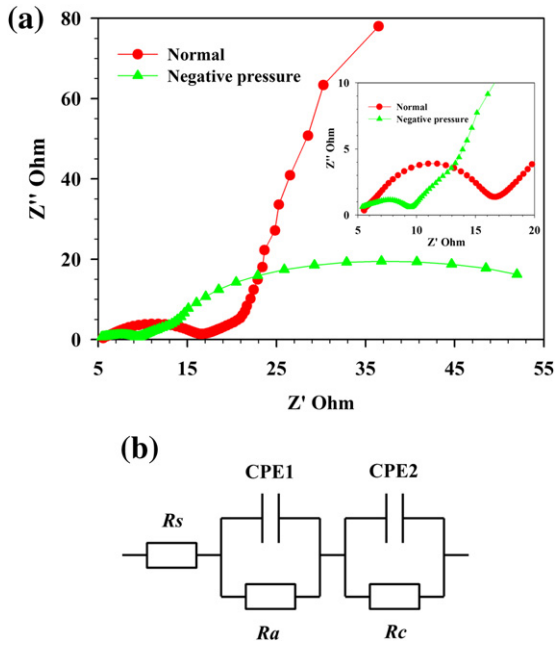

Fig. 3. (a): EIS of MFC in normal (circle) and −0.05 MPa negative pressure (triangle) environments; (b): the equivalent circuit used for data fitting.

CO_2, yielded from the metabolism of organic substances, will release from the biofilm more easily due to the decrease of partial pressure in surrounding environment. The decreased dissolved CO_2 may ease microbes from the suppression of acidification and sequentially increase the electrogenic ability of biofilm. Moreover, the faster CO_2 releases from the bulk solution, the more vigorously the solution is agitated, which may facilitate the mass transfer and reduce the R_{con}. However, the agitation might have a limited effect on reducing R_a. because the voltage did not decrease but kept stable after the MFCs being taken out from the negative pressure environment (Fig. S2). Therefore, the decrease of R_{act} must be the major reason for improving the performance of biofilm anode [3].

After being taken out from negative pressure environment, the output of MFCs didn't decrease, hence the work provides a new method to promote performance of MFCs, i.e. to firstly achieve a high power generation in negative pressure environment, after that operating the MFCs in normal environment.

3.4. Bacteria community analysis

R_a depends on the nature of the electrode materials, reactant activities, electrolyte, biofilm structure, bacterial species and their metabolisms as well as operational conditions e.g. temperature [3]. In MFCs, microbes play an important role in the electrogenesis and affect the R_a. We therefore applied DGGE to investigate the bacterial communities with different conditions as shown in Fig. 4. The profiles showed a high similarity between the three samples. Only 9 unique bands were not detected on all the three lanes (Fig. 4, labeled with arrows), and furthermore, these 9 bands were relatively weak which meant that these species had a small biomass in the biofilm. The results indicated that negative pressure only had a little effect on the diversity of bacterial community during the culture.

3.5. Morphology analysis

Marsili et al. [17] reported that biofilm on an electrode surface contributed the major current of direct electron transfer. Extracellular polymeric substances (EPSs), the important component of

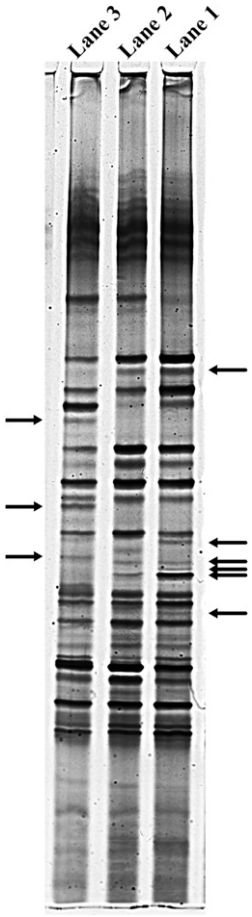

Fig. 4. DGGE profiles of bacterial communities in the anode of MFC in normal and negative pressure environments. Lane 1: anode in normal environment for two days before being placed in negative environment; lane 2: anode after being cultured in negative environment for 30 days; lane 3: anode in normal environment 20 days after being taken out from negative environment.

biofilm, are high-molecular weight compounds secreted by microorganisms and have a significant influence on the physicochemical properties including electron transfer [18]. To further investigate the mechanism, we studied the effect of negative pressure on the formation and structure of biofilm with SEM and microscopy. Two setups were cultured in normal and −0.05 MPa environments for 20 days, respectively. Although the images of microscopy showed similar morphology between the biofilm from two environments (Fig. S3), the SEM presented different results (Fig. 5). Little biofilm were observed on the surface of carbon felt that was placed in normal environment (Fig. 5a and b). In contrast, when it was cultured in −0.05 MPa, EPSs seemed to be the main component of the biofilm which tightly associated with the carbon felt and led to a rough surface (Fig. 5c and d). Except for the difference in ambient atmospheric pressure, the two MFCs were inoculated with the same sludge and fed with the same medium and the samples for SEM and microscopy were treated with the same protocols. The results indicated that negative pressure facilitated EPS adhere to electrodes and sequentially strengthen the contact between bacteria and electrode.

EPSs are composed of polysaccharides, proteins, lipids, humic-like substances and nucleic acids [18], while proteins are the predominant components in many engineered biofilm systems [19], and certain proteins appear to enhance the conductive properties of the biofilm matrix [20,21]. Therefore, the more tightly extracellular polymeric substances adhered to carbon felt, the

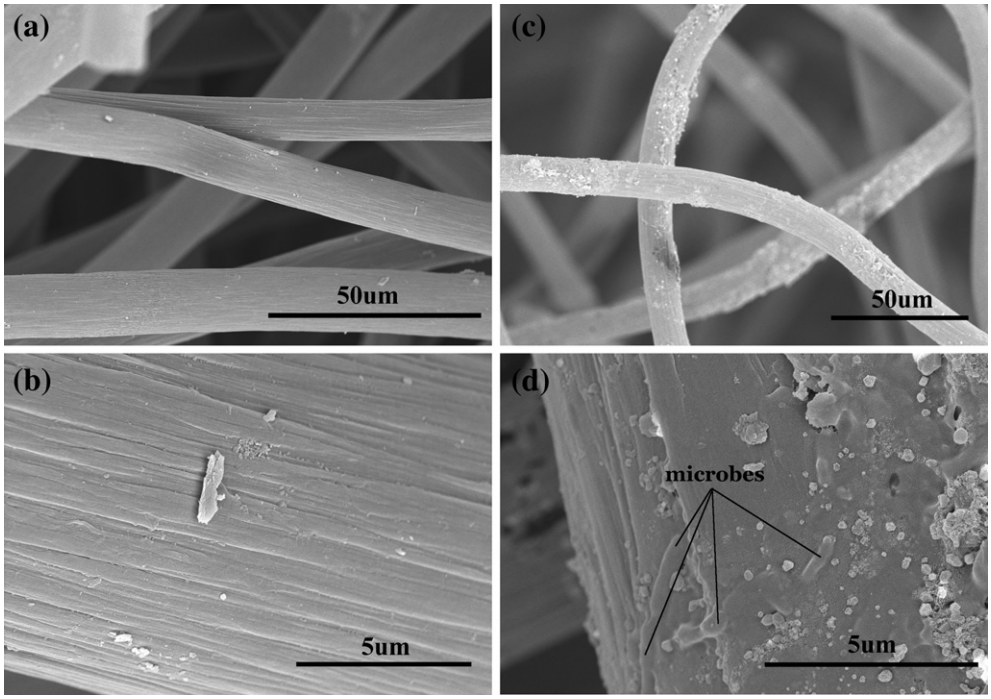

Fig. 5. SEM images of the carbon felt for culturing the biofilm in normal environment (a and b) and in −0.05 MPa environment (c and d), respectively.

lower the R_{act} might be. Since the carbon felt has been tightly packed with extracellular polymeric substances during the culture in negative pressure environment, the MFCs can maintain a low resistance and high power output. Therefore, we concluded that the promotion of negative pressure on the performance of MFCs could be mainly ascribed to EPSs. Due to the high complexity of EPSs, how they interact with the bacteria and electrodes is still under research. One possible reason for the high power output may be from some redox proteins/mediators exist in EPSs, which may broaden the scope of electron transfer for bacteria.

4. Conclusions

(1) Compared with the performance of MFCs in normal environment, the maximum power output was increased 7 times by the negative pressure. These MFCs maintain a stable ability for high electricity production in normal environment after being taken out of negative pressure environments.
(2) Though negative pressure had a little effect on the diversity of bacteria communities, it strengthened the attachment between EPSs and electrodes, and might improve the electrogenesis of biofilm. The study suggested negative pressure-culture as a method for promoting the performance of MFCs.
(3) The positive effect of negative pressure on the adhesion between biofilm and attachment materials (electrodes in the present study) also provides a potential method to improve the performance of biofilm-based technology that has been extensively applied to wastewater treatment and other fields.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpowsour.2012.11.139.

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